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(54) Title: ANTIMICROBIAL ENZYMES IN ANIMAL FEED

(57) Abstract

The use of two antimicrobial enzymes for use in feed for monogastric or non-ruminant animals is disclosed to improve growth and feed conversion ratio of poultry, pigs, veal calves and fish. This may enable the farmer to avoid the use of growth promoting antibiotics. The enzymes have antibacterial activity and one can disrupt the cell wall of bacteria (eg. lysozyme) with another producing a compound toxic to bacteria (eg. oxidase). The efficacy of these enzymes is enhanced by the inclusion of a polyunsaturated fatty acid (PUFA) such as arachidonic acid.

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ANTIMICROBIAL ENZYMES IN ANIMAL FEED

Field of the invention

This invention relates to the use of antimicrobial enzymes, such as oxidases and lysozyme, and a polyunsaturated fatty acid (PUFA), in (monogastric and non-ruminant) animal feed to improve growth and feed conversion ratio of pigs, poultry, fish and veal calves.

Background of the invention

Animals such as pigs, poultry, veal calves and fish are grown intensively for the production of meat, fish and eggs. These animals are fed diets containing a variety of raw materials of animal and/or vegetable origin to supply energy and protein. Most of the feed that is consumed is produced commercially by the compound feed industry but a significant part is produced on the farm and fed directly. The feed is supplemented with vitamins and minerals to meet the requirements of animals for these essential nutrients. In the case of poultry, the feed is also supplemented with coccidiostats to prevent coccidiosis. The use of industrially produced enzymes as feed additives has become almost common practice. Examples of such enzymes comprise phytases, alpha-amylases, proteases and various plant cell wall degrading enzymes such as ß-glucanases, endoxylanases and mannanases.

These enzymes are used to improve growth and feed conversion ratio and to reduce the environmental pollution caused by manure from pigs, poultry and fish. However, feed costs are the most important cost factor in animal production.

During the 1950's it was realized that the addition of small amount of antibiotics to animal feed resulted in improved zootechnical results in monogastric animals. Nowadays, antibiotics are used routinely as feed additives. The mode of action of these antibiotics on the improvement of growth and feed conversion ratio is still not fully understood. The generic term for this class of feed additives is growth promoters. Examples of growth promoters include virginiamycin, tylosin, flavomycin and

avoparcin.

The resistance of human pathogenic bacteria against antibiotics has been increasing rapidly. This has made it more difficult to cure people from bacterial infections. The widespread use of antibiotics in animal feed has been blamed by various experts to accelerate the build-up of resistance to various antibiotics. This has led to a ban on the use of all antibiotics as growth promoters in animal feed in Sweden and for specific antibiotics, such as avoparcin, in Denmark. It is likely that other countries will follow these examples due to pressure from consumer and health care organizations. The feed industry therefore is very much interested in natural additives with growth promoting effects without any therapeutical use in humans.

Certain enzymes are known to be active as antimicrobial agents, and these may be used in the preservation of food. Glucose oxidase has also been suggested for the preservation of silage fodder and silage grain (WO-A-98/01694, Suomen Sokeri Oy).

Plant cell wall degrading enzymes such as mannanase and b-glucanases are used as feed additives for diets containing high amounts of b-glucan and mannan to reduce the viscosity in the gastro-intestinal tract of monogastric animals caused by these non-starch polysaccharides. These enzymes also have some antifungal activity but do not exhibit any antibacterial activity.

The antibacterial enzyme lysozyme has been added as a growth promoter to the feed in monogastric animals (Latvietis, J., *et al*, In: Vitamine Zusatzstoffe in der Ernaehrung von Mensch und Tier. Symposium 5th (1995). Editor Rainer Schubert et al. Jena, September 28-29. ISBN 3.00.000361-4). These authors added lysozyme prepared from egg white to the feed of broilers and veal calves. Growth and feed conversion were allegedly improved. The concept however of using mixtures of antibacterial enzymes in combination with enzyme enhancers (eg. PUFAs) has not been published.

It is thus desirable for farmers and the compound feed industry to obtain an optimum growth and feed conversion ratio, at minimum cost, in a sustainable way, respecting demands from both consumer and health care organisations alike.

Description of the Invention

The present invention provides an animal feed additive composition comprising a

mixture of antimicrobial enzymes which can show synergistic effects. The effect of the or each enzyme can be enhanced by the presence of one or more PUFAs. This may allow the improvement of growth and feed conversion ratio of animals such as pigs, poultry, veal calves and aquatic or marine animals such as fish, and can allow one to omit an antibiotic as growth promoter.

A first aspect of the present invention relates to an animal feed additive composition, suitable for a monogastric or non-ruminant animal, the composition comprising at least two antimicrobial enzymes and (as an enzyme enhancer) a polyunsaturated fatty acid (PUFA).

Preferably one or two of the enzymes are antibacterial enzymes. These enzymes may be of different types and/or may have different activity. One, eg. a first, enzyme may be able to disrupt the cell wall of bacteria. The enzyme may be one that can attack or degrade peptidoglycans. For example, the enzyme may be able to cleave off peptidoglycans. A preferred enzyme for this task is lysozyme. This (first) enzyme may be present at a concentration to give from 50,000 to 150,000, such as from 70,000 to 130,000, optimally from 90,000 to 110,000 Shugar units per kilogram (or unit) of animal feed. In terms of weight, therefore, this first enzyme may be present at an amount to give a concentration in the animal feed of from 1 to 8 grams per kg of feed, preferably from 2 to 7 grams per kg of feed and optimally from 3 to 5 grams per kg of feed.

The second enzyme may be able to generate a compound that is toxic to the bacteria. This may be the same bacteria, of different, from the bacteria whose cell walls can be disrupted or degraded by the first enzyme. The compound is preferably a peroxide, eg. hydrogen peroxide. Thus preferred enzyme are oxidases. Particularly preferred is glucose oxidase. This second enzyme may be present at a concentration to give from 500 to 1,500, preferably from 700 to 1,300, and optimally from 900 to 1,100 Sarett U per kilogram (or unit) of feed. Thus preferably this second enzyme may be present at an amount, by weight, to give a final concentration in the animal feed of from 1 to 8 grams per kg of feed, preferably from 2 to 7 grams per kg of feed, and optimally from 3 to 5 grams per kg of feed.

Enzymes can function as antimicrobial agents in the following ways:

a) disruption of the cell wall;

b) generation of a toxic compound;

c) removal of an essential nutrient; or

d) inactivation of enzymes essential for growth.

Each of these will be discussed in turn.

a) Microbial cell walls vary in structure for fungi, yeasts, gram negative and gram positive bacteria. One can need different enzymes to disrupt the cell wall of these different types of microorganisms. The fungal and yeast cell wall, for example, may be disrupted by mannanases, chitinases and betaglucanases. The bacterial cell wall, however, is not sensitive to these enzymes due to a different type of structure. Gram positive organisms have a peptidoglycan layer covered by some protein but essentially consists of peptidoglycan only. This substrate may be degraded by action of lysozyme (1,4-b-acetylmuramidase) which cleaves peptidoglycans between the C1 of N-acetyl-muramic acid and the C-4 of N-acetylglucosamine.

The peptidoglycan layer is covered by a tight lipopolysaccharide-protein-divalent cation-phospholipid layer in gram negative bacteria. This layer can hinder the efficacy of lysozyme in gram negative bacteria. Agents capable of disrupting this tight lipopolysaccharide layer stimulate the action of lysozyme by giving the enzyme access to the peptidoglycan layer.

b) Oxidases are capable of producing hydrogen peroxide which is lethal to most microorganisms. Glucose oxidase , for example, catalyses the conversion of glucose into gluconic acid and hydrogen peroxide. Xanthine oxidase, present in milk, is also capable of generating hydrogen peroxide.

Other antimicrobial compounds which may be enzymatically generated comprise hypothiocyanate (produced by lactoperoxidase), chloramines (produced by myeloperoxidase), free fatty acids (produced by lipase), poly-unsaturated fatty acids, lysophosphatidylcholine (produced by phospholipase A2) and xylitol-5-phosphate (produced by xylitol phosphorylase). This list is by no means exhaustive, however.

c) Oxygen may be removed from the media by means of oxidases such as e.g. glucose oxidase. Complete removal of oxygen prevents the growth of aerobic microorganisms.

d) Enzymes essential for growth of microorganisms may be inactivated by means of other enzymes. Sulfhydryl oxidases, for example, are capable of inactivating

enzymes which depend on active sulfhydryl groups for their activity.

The composition can comprise or enzyme enhancer, such as a compound that can significantly improve the activity of the or each antimicrobial (eg. antibacterial) enzyme, preferably in a synergistic manner. The enzyme enhancer can comprise one or more polyunsaturated fatty acids, otherwise known as PUFAs. The or each PUFA can be of the n-3 or n-6 family. Preferably it is a C18, C20 or C22 PUFA. The PUFA can be provided in the form of a free fatty acid, as a fatty acid ester (e.g. methyl or ethyl ester), as a phospholipid and/or in the form of a triglyceride.

Preferred PUFAs include arachidonic acid (ARA), docosohexaenoic acid (DHA), eicosapentaenoic acid (EPA) and/or γ-linoleic acid (GLA). Of these, ARA is preferred.

The PUFAs may be from a natural (e.g. vegetable or marine) source or may be derived from a single cell or microbial source. In particular, the PUFA may be produced by a bacteria, fungus or yeast. Fungi are preferred, preferably of the order *Mucorales*, for example *Mortierella*, *Pythium* or *Entomophora*. The preferred source of ARA is from *Mortierella alpina* or *Pythium insidosum*.

The PUFA may be present as an oil. Suitable oils that include ARA are available from DSM N.V., Wateringseweg 1, P.O. Box 1, 2600 MA Delft, The Netherlands, under the trade mark VEVODAR™. Another commercially available oil is ARASCO™ from Martek Corporation, 6480 Dobbin Road, Columbia, MD 21045, United States of America. Other PUFAs are available, for example DHA as a DHA oil (DHASCO™ from Martek Corporation or DHA from Pronova, Norway, under the trade mark EPAX™).

The PUFA is preferably at a concentration that it allows it to be added to the animal feed to give a final concentration of from 0.1 or 1 to 1000, such as from 0.5 to 50 or 1 to 100, and preferably from 1 to 10 grams per kilogram (or unit) of feed.

All the antimicrobial enzymes can be produced on industrial scale and/or may be recombinant. Lysozyme is commercially available, isolated from egg white, or may be recombinant. The or each enzyme may be naturally occurring or may be an (eg. recombinant) variant or mutant thereof.

The or each antibacterial enzyme is preferably recombinantly produced such as by expression of a heterologous gene or cDNA in a suitable organism, or alternatively by

homologous (over)expression of a suitable endogenous gene. The glucose oxidase gene, for example, has been overexpressed in recombinant systems (WO-A- 89/12675, Chiron). Lysozyme (from egg white) can be recombinantly expressed by expression of the gene in *Aspergillus niger* (Archer, D.B. *et al.*, Bio/Technology 8: 741-745 (1990). Lysozyme mutants (produced by protein engineering) can also be used which have better heat stability and stronger antimicrobial action.

The antimicrobial enzymes used in the invention will usually be either ones which are not a natural constituent of the animal feed or are present in the feed at a concentration different from its natural concentrations.

A second aspect of the present invention relates to an animal feed composition comprising at least two antimicrobial enzymes and a PUFA. As with the additive composition, a first enzyme may be able to disrupt the cell wall of bacteria, and a second enzyme may be capable of generating a compound toxic to the bacteria.

A third aspect of the invention relates to a process for the preparation of an animal feed composition, the process comprising adding to one or more edible feed substance(s) or ingredient(s) two or more antimicrobial enzymes and a PUFA, or an additive of the first aspect.

The enzymes and/or PUFA can be added to the animal feed composition separately from the feed substance(s) or ingredient(s), individually or in combination with other feed additives. Alternatively, or in addition, the or each enzyme can be an integral part of one of the feed substances. This aspect includes both preparing a feed composition with the two enzymes and PUFA or supplementing an existing feed composition with these compounds.

A particularly preferred method for the (exogenous) addition of the antimicrobial enzyme to animal feed is to add the or each enzyme as transgenic plant material and/or (e.g. transgenic) seed. The enzyme may thus have been synthesized through heterologous gene expression, for example the gene encoding the desired enzyme may be cloned in to a plant expression vector, under control of the appropriate plant expression signals, e.g. a tissue specific promoter, such as a seed specific promoter. The expression vector containing the gene encoding the enzyme can be subsequently transformed into plant cells, and transformed cells can be selected for regeneration into whole plants. The thus obtained transgenic plants can be grown and harvested, and those parts of the plants

containing the heterologous (to the plant) enzyme can be included in one of the compositions, either as such or after further processing. Reference here is made to WO-A-91/14772 which discloses general methods for the (heterologous) expression of enzymes in (transgenic) plants, including methods for seed-specific expression of enzymes. The heterologous enzyme may be contained in the seed of the transgenic plants or it may be contained in other plant parts such as roots, stems, leaves, wood, flowers, bark and/or fruit.

The addition of the enzyme in the form of transgenic plant material, e.g. transgenic seed containing the antimicrobial enzymes, may require the processing of the plant material so as to make the enzyme available, or at least improve its availability. Such processing techniques may include various mechanical (eg. milling and/or grinding) techniques or thermomechanical treatments such as extrusion or expansion.

The antibacterial enzymes may be added to the feed composition at a concentration which varies as a function of diet composition, type of enzyme and target animal species.

Preferably the compositions of the invention do not contain any antibiotics. The composition(s) of the invention may also be free of a mineral component (such as zinc and/or iodine) and/or an immunomodulating agent (such as ascorbic acid). Although each of the two antimicrobial enzymes and the PUFA can all be produced by a micro-organism, it is preferred that the composition is free of microorganisms that produced any of these compounds (or microorganisms from *Streptomyces*). Furthermore, the composition may be devoid of microorganisms that produce lactic acid inside the animal (eg. those of the genus *Lactobacillus* or *Enterococcus*).

A fourth aspect of the present invention relates to a process for promoting growth and/or feed conversion in a monogastric or non-ruminant animal, the process comprising feeding the animal at least two antimicrobial enzymes and a PUFA or a feed composition of the first or second aspect or preparable by the third aspect.

Suitable animals include farm, monogastric and/or non-ruminant animals such as pigs (or piglets), poultry (such as chickens, turkeys), calves or veal or aquatic (e.g. marine) animals (for example fish).

A fifth aspect relates to the use of a composition of the first aspect as an additive for a monogastric or non-ruminant animal feed composition.

Preferred features and characteristics of one aspect of the present invention are applicable to another aspect *mutatis mutandis*.

The present invention will now be described by way of example with reference to the following Examples which are provided by way of illustration and are not intended to limit its scope.

Comparative Examples 1 to 4 and Example 5

Characterization of antibacterial enzyme products

Glucose oxidase (EC 1.1.3.4), an oxidase capable of generating hydrogen peroxide, was obtained as a commercial product under the trade mark FERMIZYME GO™ from DSM/Royal Gist-brocades, Bakery Ingredients Division, PO Box 1, 2600 MA DELFT, The Netherlands. This enzyme preparation exhibits an activity of 500 Sarett Units per gram. One Sarett unit is the amount of enzyme that will cause an uptake of $10mm^3$ of oxygen per minute in a Warburg manometer at 30°C in the presence of excess oxygen and 3.3% glucose monohydrate in a phosphate buffer pH 5.9. The enzyme was produced by the fungus *Aspergillus niger*.

Lysozyme obtained from chicken egg-white was obtained as a commercial product under the trade mark DELVOZYME™ from DSM/Royal Gist-brocades, Dairy Ingredients Group, PO Box 1, 2600 MA DELFT, The Netherlands. The product contains 5.1×10^6 Shugar units/ml product. One Shugar unit is defined as the amount of enzyme which causes a decrease of absorbance of 0.001 per minute at 450 nm and pH 6.2 at 25°C in a suspension of *Micrococcus lysodeikticus* (0.25 mg/ml) obtainable from Sigma Chemicals.

Characterization of arachidonic acid

Arachidonic acid (ARA) was also obtained from DSM/Royal Gist-brocades under the trade mark VEVODAR™. This is in the form of a microbial oil (ARA content at least 35%) obtained by culturing the fungus *Mortierella alpina*.

Application of antibacterial enzymes in animal feed for poultry

Trials we carried out with broilers to test the efficacy of glucose oxidase and lysozyme alone and the combination of both. Male broilers (Ross) we kept from day 1

to day 5 on a standard diet. At day 5, animals we selected from this group and are divided into cages. The weight of the animals and their variation were measured. The average weight and its deviation were equal per cage. Fifteen animals were kept in one cage. The cages were situated in an artificially heated, ventilated and illuminated broiler house. The floor space of each cage was 0.98 m^2, with wire floors. The broiler house was illuminated for 24 hours per day. During the experimental period, light intensity was gradually reduced. The temperature was gradually reduced from 28°C during the first week to 23°C during the last week of the experiment. Humidity in the broiler unit was approximately 60% during the experimental period. The animals had been vaccinated against New Castle disease (using the spray method) at an age of one and fourteen days. The experiment lasted 33 days, comprising a pre-test period of 5 days and a test period of 28 days.

The experimental diets were offered *ad lib.* to the animals. Water was freely available. The feed was cold pelleted (temperatures were kept below 65°C) at a diameter of 3 millimeter.

The experiment comprised the following treatments:

1) basal diet (negative control)
2) basal diet + glucose oxidase (1000 Sarett U/kg feed)
3) basal diet + lysozyme (100.000 Shugar units/kg of feed)
4) basal diet + glucose oxidase (1000 Sarett U/kg of feed) + lysozyme (100.000 Shugar units/kg of feed)
5) basal diet + glucose oxidase (1000 Sarett U/kg of feed) + lysozyme (100,000 Shugar units/kg of feed) + arachidonic acid (ARA) to a final concentration of 1 g/kg of feed.

Each treatment was replicated six times (90 birds per treatment in total). Gain and feed conversion were measured. The composition of the feed (basal diets) used was:

Ingredients	Content (%)
Rye	10
Wheat	40
Soy oil	1
Animal fat	6

Manioc	4.3
Soya bean meal (45.4% crude protein)	22
Full fat toasted soya beans	10
Meat meal tankage (58% crude protein)	3
Vitamins/premix	1
Limestone	0.9
Monocalciumphosphate	1.2
Salt (NaCl)	0.3
D,L-methionine	0.2

ME broilers (KCal/kg)	2850
Crude protein (%)	21.4
Crude fat (%)	10.5
Lysine (available, %)	1.23 (1.04)
Methionine + cysteine (available, %)	0.90 (0.79)

The enzymes and arachidonic acid were added to this basal diet by mixing it first with a carrier.

The effects of the antibacterial enzymes and arachidonic acid on growth and feed conversion ratio in broilers between 5 and 33 days of age are shown below in Table 1.

TABLE 1

Example	Diet	Feed Intake (g)	Growth (g)	Feed conversion ratio	Improvement in feed conversion ratio
1	Basal diet	2,760	1,540	1.79	-
2	Basal diet + glucose oxidase	2,750	1,554	1.77	-0.02
3	Basal diet + lysozyme	2,748	1,553	1.77	-0.02
4	Basal diet + glucose oxidase + lysozyme	2,731	1,589	1.72	-0.05

5	Basal diet + oxidase + lysozyme + arachidonic acid	2,710	1,595	1.70	-0.09

The addition of one type of antibacterial enzyme or a combination of different types of antibacterial enzymes both improved the growth and feed conversion ratio in broilers. However, more importantly a synergistic effect was found for the combination of glucose oxidase and lysozyme on the feed conversion ratio and the inclusion of arachidonic acid in diets containing antibacterial enzymes resulted in an even further improvement.

Comparative Examples 6 to 9 and Example 10

Application of antibacterial enzymes in animal feed for pigs

Crossbred pigs (equal barrows and gilts; n = 100) of a similar age and weight were used in this trial. They were housed in environmentally controlled rooms, and had *ad lib.* access to feed and water at all times. The room temperature was set initially at 29°C and was lowered about 2°C per week after the second week. The pigs were allotted to one of five treatments. There were two pigs in each pen with 10 replications (weight blocks) per treatment.

Body weight and pen feed consumption were measured weekly.

The basal diet was a typical American diet, of the composition:

Raw Material	Content (%)
Corn	63.6
Soyabean meal	30.9
Vitamin premix	0.25
Trace mineral premix	0.1
Selenium premix	0.05
Dicalcium phosphate	1.2
Salt (NaCl)	0.3
Limestone	3.6

No antibiotic was added to the feed.

The experiment comprised the following treatments (Examples 6 to 10):

a) basal diet (negative control);

b) basal diet + glucose oxidase (1000 Sarett U/kg feed);

c) basal diet + lysozyme (100,000 Shugar units/kg of feed);

d) basal diet + glucose oxidase (1000 Sarett U/kg of feed) + lysozyme (100,000 Shugar units/kg of feed);

e) basal diet + glucose oxidase (1000 Sarett U/kg of feed) + lysozyme (100,000 Shugar units/kg of feed) + arachidonic acid to a final concentration of 1 g/kg of feed.

The results obtained in terms of feed intake, growth and feed conversion ratio are shown below in Table 2.

TABLE 2

Effects of antibacterial enzymes and ARA on growth and feed conversion ratio in growing pigs (23 to 54 kg body weight).

Example	Diet	Daily Feed Intake (g)	Daily gain (kg)	Feed conversion ratio	Improvement in feed conversion ratio
6	Basal diet	2.20	0.90	2.44	-
7	Basal diet + glucose oxidase	2.15	0.90	2.39	-0.05
8	Basal diet + lysozyme	2.14	0.89	2.40	-0.04
9	Basal diet + glucose oxidase + lysozyme	2.10	0.94	2.23	-0.21
10	Basal diet + oxidase + lysozyme + arachidonic acid	2.05	0.95	2.16	-0.28

The addition of one type of antibacterial enzyme or combinations of different types of antibacterial enzymes showed a favourable effect on daily gain and feed

conversion ratio. However, the combination of two different types of antibacterial enzymes (i.e. glucose oxidase and lysozyme) resulted in a surprising synergistic effect on feed conversion ratio and the addition of arachidonic acid to feed containing antibacteria enzymes resulted in a further improvement.

Comparative Examples 11 to 14 and Example 15

The use of antibacterial enzyme in fish nutrition

Effects of supplemental antibacterial enzymes on growth and feed conversion ratio were studied with trout (*Oncorhynchus mykiss*).

The diet composition used was as follows:

Raw material	Content (%)
Soyabean meal	43
Soya beans, pressure cooked	20
Wheat gluten	20.5
Fish oil	12
L-lysine-HCl	0.8
D, L-methionine	0.2
Vitamin/mineral premix	3.5

No growth promoting antibiotic was added to the feed.

Experiments were conducted with 200 trout with a mean initial body weight of 8.8 g/trout which were allotted to 5 equal groups. Diets were fed to these 5 groups over a period of 53 days. The water temperature was kept constant at 15°C. The diets were fed twice daily to satiation avoiding feed losses. Weight gain and feed conversion ratio were determined.

The experiment comprised the following treatments (Examples 11 to 15):

a) basal diet (negative control);

b) basal diet + glucose oxidase (1000 Sarett U/kg feed);

c) basal diet + lysozyme (100,000 Shugar units/kg of feed);

d) basal diet + glucose oxidase (1000 Sarett U/kg of feed) + lysozyme (100,000 Shugar units/kg of feed); and

e) basal diet + glucose oxidase (1000 Sarett U/kg of feed) + lysozyme (100,000 Shugar units/kg of feed) + arachidonic acid to a final concentration of 1 g/kg of feed.

The results obtained, in terms of growth and feed conversion, are shown below in Table 3.

TABLE 3

Gain, feed intake and feed conversion ratio in trout fed for 53 days on diets supplemented with antibacterial enzymes +/- arachidonic acid.

Example	Diet	Feed Intake (g/trout)	Gain (g/trout)	Feed conversion ratio	Improvement in feed conversion ratio
11	Basal diet	18.5	12.5	1.48	-
12	Basal diet + glucose oxidase	20.6	14.1	1.46	-0.02
13	Basal diet + lysozyme	20.4	14.1	1.45	-0.03
14	Basal diet + glucose oxidase + lysozyme	21.5	16.4	1.31	-0.17
15	Basal diet + oxidase + lysozyme + arachidonic acid	22.9	18.4	1.24	-0.24

The results obtained demonstrate the favourable effects of one type of antibacterial enzyme or a combination of antibacterial enzymes on growth and feed conversion ratio in trout. The combination of different types of antibacterial enzymes showed a synergistic effect on feed conversion ratio and the addition of arachidonic acid to diets containing antibacterial enzymes gave a further improvement.

CLAIMS

1. An animal feed additive composition comprising at least two antimicrobial enzymes and a polyunsaturated fatty acid (PUFA).

2. An animal feed composition comprising at least two antimicrobial enzymes and a polyunsaturated fatty acid (PUFA).

3. A composition according to claim1 or 2 wherein the or each antimicrobial enzyme is an antibacterial enzyme.

4. A composition according to claim 3 wherein one or more of the antibacterial enzymes comprises glucose oxidase, sulphydryl oxidase, xanthine oxidase, peroxidase or a lysozyme.

5. A composition according to claims 1 or 2 wherein one of the enzymes is able to disrupt the cell wall of bacteria and/or another enzyme is capable of generating a compound that is toxic to the bacteria.

6. A composition according to any preceding claim wherein the enzymes are a lysozyme and an oxidase.

7. A composition according to any preceding claim wherein the PUFA comprises an n-3 or n-6 C18, C20 or C22 PUFA.

8. A composition according to any preceding claim wherein the PUFA is in the form of a free fatty acid, fatty acid ester, phospholipid or triglyceride.

9. A composition according to any preceding claim wherein the PUFA comprises arachidonic acid (ARA).

10. A composition according to any preceding claim wherein the or each antibacterial enzyme is derived from an animal, an animal product, a plant or a microorganism.

11. A composition according to any preceding claim wherein the or each antibacterial enzyme is of microbial origin and/or is a recombinant protein.

12. A composition according to any preceding claim wherein the or each enzyme is derived from, produced by or present in a microorganism such as a bacteria, yeast or (filamentous) fungus.

13. A composition according to claim 11 wherein the microorganism is of the genus *Streptomyces, Bacillus, Escherichia, Saccharomyces, Kluyveromyces, Hansenula, Pichia, Yarrowia, Candida, Aspergillus, Trichoderma, Penicillium, Mucor, Fusarium* or *Humicola.*

14. A composition according to claim 13 wherein the microorganism is *Streptomyces lividans, Escherichia coli, Bacillus licheniformis, Kluyveromyces lactis Aspergillus niger*, or *Mortierella alpina*.

15. A composition according to any preceding claim wherein the enzyme is contained in plant material, optionally obtained from a transgenic plant.

16. A composition according to claim 15 wherein the antibacterial enzymes glucose oxidase and/or lysozyme are contained in seeds of a transgenic plant.

17. A composition according to any preceding claim which is adapted to comprise from 10 to 10,000 Sarett Units of glucose oxidase per kg feed and 1000 to 1,000,000 Shugar units of lysozyme per kg feed.

18. A composition according to any preceding claim which comprises 1-1000 g of arachidonic acid per kg of feed.

19. A process for the production of a feed composition for a monogastric or non-ruminant animal, the process comprising adding two antimicrobial enzymes and a PUFA to, or mixing a feed additive composition according to any of claims 1 or 3 to 18 with, one or more edible feed substance(s) or ingredient(s).

20. An animal feed composition comprising an additive composition according to any of claims 1 or 3 to 18 and one or more edible feed substance(s) or ingredient(s).

21. A process for promoting growth and/or feed conversion in a monogastric or non-ruminant animal, the process comprising feeding the animal at least two antimicrobial enzymes and a PUFA or a composition as defined in any of claims 1 to 18 or 20.

22. A process according to claim 21 wherein the animal is a pig, poultry (chicken, turkey), veal or aquatic animal.

23. The use of a composition according to any of claims 1, 3 to 18 as an additive for a monogastric animal feed composition.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/07835

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23K1/16 A23K1/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 15682 A (ERBER ERICH KG) 30 May 1996 (1996-05-30) page 3, line 17 -page 4, line 30 page 6, line 11 - line 15 page 7, line 4 - line 27 example claims 1-6	1-10, 19-23
Y	EP 0 366 869 A (LYCON AG) 9 May 1990 (1990-05-09) page 2, line 42 - line 54 page 3, line 19 - line 34 page 5, line 15 - line 17 claims 1,2,7,15,16,22-25	1-10, 19-23

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

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Date of the actual completion of the international search	Date of mailing of the international search report
1 February 2000	09/02/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Dekeirel, M

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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	EP 0 372 502 A (HOECHST AG) 13 June 1990 (1990-06-13) page 3, line 19 - line 27 example 6 claims 1,2.9,10 ---	1,2, 19-23
A	WO 97 29645 A (BIOFEED THAILAND CO LTD ;BUNKE PATRIK (SE); LINDBLOM RAGNVALD (TH)) 21 August 1997 (1997-08-21) claims 1,3,10,12-20,24 -----	1,2, 19-23

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